Monoamine Oxidase-B: Alternative Target of [18F]THK-5351

VI. 6. Monoamine Oxidase-B: Alternative Target of [^{18}F]THK-5351

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Introduction

[^{18}F]THK-5351 was one of first generation tau PET tracers that was designed originally to detect tau aggregates in the form of paired helical filaments found in brains of patients with Alzheimer's disease (AD). Previous in vitro autoradiography of [^{18}F]THK-5351 exhibited the selective binding in a laminar fashion in the cortex of formalin fixed brain sections from AD, which corresponded to tau immunohistochemistry[1,2]. Clinical PET studies of [^{18}F]THK-5351 demonstrated that elevated tracer retention in sites susceptible to tau deposition in various tauopathies including AD, progressive non-fluent aphasias (PNFA), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS)[2-4]. However, there was high binding of [^{18}F]THK-5351 in the subcortical regions such as the basal ganglia and thalamus even in control subjects whose brains are not expected to harbor tau aggregates. In addition, the tracer binding in these regions was correlated with age. To understand the binding nature of [^{18}F]THK-5351 and identify its targets, we investigated the binding property of [^{18}F]THK-5351 using fresh frozen human brain tissues including an autopsy-confirmed case of AD who underwent [^{18}F]THK-5351 PET scan before death.

Methods

Experiments were performed under the regulations of the Ethics Committee of the Tohoku University Graduate School of Medicine. Postmortem brain tissues from control subjects and patients with AD were acquired from Tohoku University Brain Bank and Tissue Solutions Ltd (Glasgow, UK). Twelve-micron thick brain slices were generated with a cryostat (Microm HM560; Thermo Scientific, Waltham, MA) using a -20°C chamber temperature and -15°C object temperature. Sections were transferred to MAS-coated glass

slides (Matsunami Glass Ind., Ltd, Osaka, Japan). After drying, the sections were stored at -80°C. Brain sections were dried and dipped in PBS for a total of 25 min, and then pre-incubated in PBS containing 1% bovine serum albumin (BSA). Brain sections were then incubated for 30 min at room temperature with 3 nM [^{3}H]THK-5351 (molar activity, 2.96 TBq/mmol; radiochemical purity, 98.9%, Sekisui Medical Inc., Tokyo, Japan). After incubation, sections were washed sequentially with PBS containing 1% bovine serum albumin (BSA) for 5 min, followed by PBS for 5 min twice. Dried sections were exposed to an imaging plate for tritium (BAS IP TR 2025 E, GE Healthcare, UK) for 3 days. The autoradiographic images were obtained from Typhoon FLA-7000 (GE Healthcare). *In vitro* competitive autoradiography was performed in the presence of 3 μM unlabeled ligands or inhibitors, which were obtained from Sigma-Aldrich and Tocris Bioscience (Bristol, UK).

Results

Displaceable binding of [^{3}H]THK-5351 was observed in the basal ganglia of normal subjects containing no amyloid plaques and tau tangles (Fig. 1). Competitive autoradiography was performed using various ligands whose targets have been reported in human basal ganglia. Lazabemide and rasagiline, which are known as reversible and irreversible monoamine oxidase-B (MAO-B) inhibitors, respectively, displaced [^{3}H]THK-5351 binding completely. However, no remarkable competition was observed with the other tested ligands, including a dopamine transporter inhibitor (GBR12935), μ-opioid receptor agonist (DAMGO), MAO-A inhibitor (clorygyline), and I_2 imidazoline receptor ligand (idazoxan) (Fig. 1).

In vitro autoradiography of [^{3}H]THK-5351 in various regions from an autopsy-confirmed AD case who underwent [^{18}F]THK-5351 PET scan before death demonstrated [^{3}H]THK-5351 binding was substantially reduced in the presence of MAO-B inhibitor, Lazabemide, whereas [^{3}H]THK-5351 binding remained detectable , albeit the binding in the basal ganglia was completely displaced (Fig. 2).

Discussions

This study demonstrated that tau PET tracer [^{18}F]THK-5351 bound to MAO-B as well as tau aggregates in fresh frozen human brain tissues. A recent human blocking study using selective irreversible MAO-B inhibitor, selegiline, confirmed this finding in vivo[5]. Recent imaging-autopsy correlations demonstrated that in vivo [^{18}F]THK-5351 binding was correlated with the density of tau aggregates and MAO-B in a patient with AD and PSP[6,7].

Previously, we observed selective binding to tau aggregates in paraffin-embedded fixed brain sections[1,2]. However, the fixation of tissues may affect the tracer binding because formalin produces cross-linkage of proteins and may result in diminishing the natural binding sites such as MAO-B enzymes or yielding the artificial binding sites. In fact, [^{18}F]THK-5351 binding in the basal ganglia disappeared after fixation (data not shown). These results highlighted the importance of appropriate experimental procedure in the evaluation of tracer binding.

Conclusions

MAO-B is an alternative binding target of [^{18}F]THK-5351. MAO-B is an attractive target for in vivo assessment of neuroinflammation such as astrogliosis. Therefore, [^{18}F]THK-5351 PET might be useful imaging biomarker for astrocytosis in neurodegenerative diseases.

References

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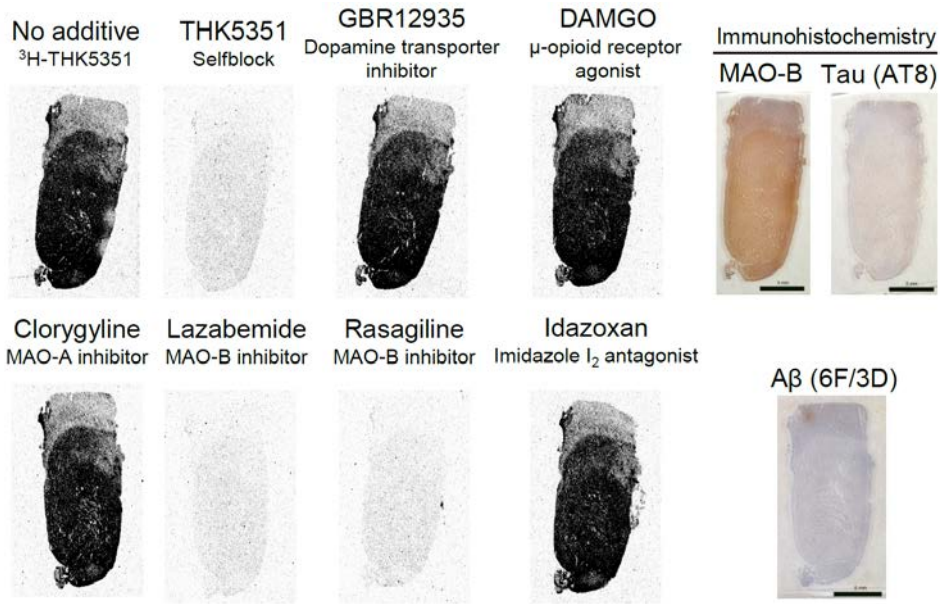

Figure 1. *In vitro* autoradiograms of [^{3}H]THK-5351 in basal ganglia brain sections from a normal control subject (83-year-old male) in the absence (no additive) and presence of unlabeled THK5351 and various inhibitors/ligands, namely GBR12935 (dopamine transporter inhibitor), DAMGO (μ-opioid receptor agonist), clorygyline (MAO-A inhibitor), lazabemide (MAO-B inhibitor), rasagiline (MAO-B inhibitor) and idazoxan (imidazoline I_2 antagonist). Immunohistochemistry images of tau (AT8), Aβ (6F/3D), and MAO-B are also shown.

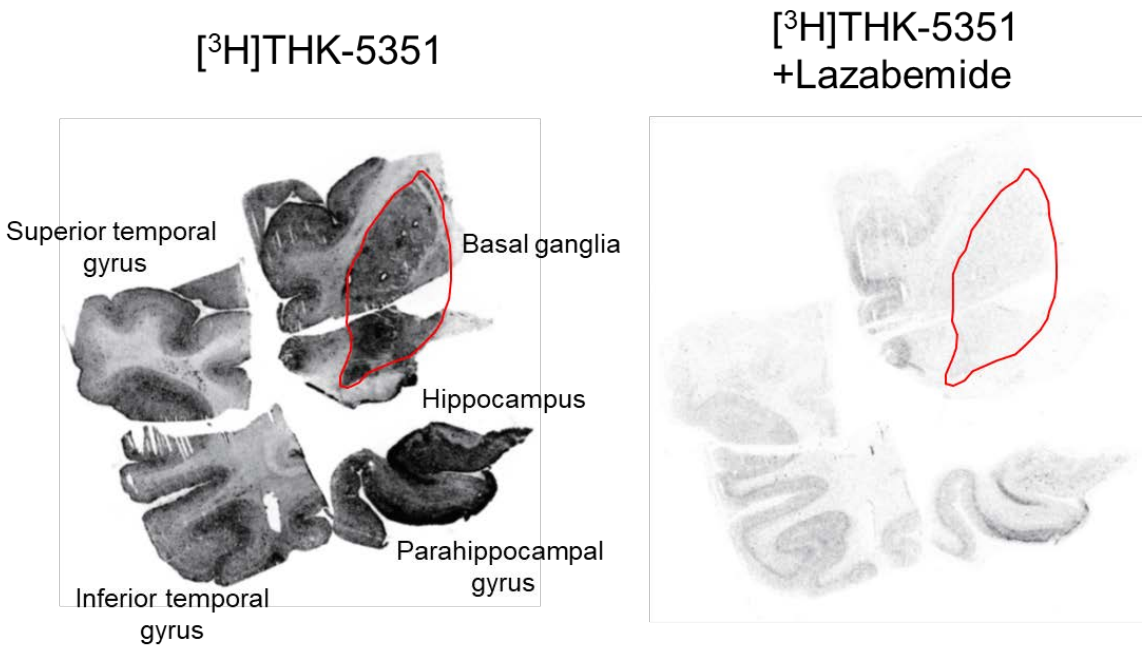

Figure 2. In vitro autoradiography of [^{3}H]THK-5351 in brain sections from an autopsy-confirmed AD case (81-y-old male) who underwent [^{18}F]THK-5351 PET scan before death in absence and presence of selective reversible MAO-B inhibitor, Lazabemide. Red notes the basal ganglia.